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Ancestry estimation of three Mediterranean populations based on cranial metrics

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Highlights

- Ancestry estimation is very difficult due to globalisation and admixture
- This paper used a custom-made approach for separating Greek-Cypriots and
- Turks
- Discriminant function analysis resulted in up to 98% cross-validation accuracy
- The results suggest that this method is useful for forensic identification in Cyprus

Abstract:

The estimation of ancestry is an essential benchmark for positive identification of heavily decomposed bodies that are recovered in a variety of death and crime scenes. This is especially true when reconstructing the biological profile of the deceased as most methods for sex, age and stature estimation are population-specific. Ancestry estimation methods

vary from traditional morphological assessment of cranial features and biometric quantification to computer-aided shape analysis and classification with specialised software. The current paper aims to explore population differences between three neighbouring countries (Greece, Cyprus and Turkey) that have been in constant interaction through conflicts and population movements from the ancient past to the present day, through cranial measurements.

The sample consists of 160 dry crania of Greek origin, 137 dry crania of Greek-Cypriot of origin Cyprus and 380 CT scans from Turkish individuals. Twelve measurements were taken in both dry and virtual skulls. Data were submitted to Principal component analysis and discriminant function analysis. Intra- and inter-observer error as well as the measurement error between virtual and physical measurements were quantified using TEM, rTEM and R.

Measurement error was very low in all cases. Classification accuracy for cross-validated data ranged from 74.1 to 97.9%. The highest accuracy was obtained for the Turkish sample both in males and females. The results are in accordance with genetic data on the three populations.

These results create great confidence in the application of the produced functions in forensic cases requiring ancestry estimation in Cyprus, specifically to unidentified individuals from the 1974 conflict. In addition, these standards can be applied in other forensic situations where ethnicity is an issue but the geographic area of origin is limited to the area encompassing Turkey, Cyprus and Greece.

Keywords: Forensic Anthropology, ancestry estimation, cranial measurements, Mediterranean populations, Forensic identification

Introduction

Population affinities and gene exchange between neighbouring populations, as well as secular changes and sexual dimorphism in populations from the same geographic areas are typically explored through a variety of biometric studies. Craniometric studies are of particularly interest in studying biological distance between populations, as they assume that observed variation is, in large part, a result of genetic factors [1,2]. In fact, recent studies affirm a correlation between craniometric features and classic genetic markers, such as DNA polymorphisms [3]. This relationship underlies the utility of craniometrics in forensic contexts where identifying population affinity is of crucial importance for establishing positive identification.

Ancestry in forensic anthropology refers to the “individual’s ancestral geographic region of origin,” [4] and it is estimated by evaluating metric and non-metric skeletal traits

that correspond to geographically-patterned genetic variation [5]. The estimation of an individual's ancestry is an essential benchmark for positive identification of unknown human remains, as it affects other methods of biological profiling such as sex and stature estimation, which are known to be population specific. In addition, the ancestry of unidentified human remains in combination with the scene evidence often defines the course of the forensic investigation as to the circumstances of the individual's death.

Common methods of ancestry estimation of the past involved the morphological analysis of traits that appear predominantly in a given ancestral group. Lists of traits thought to be more frequent in certain groups compiled by various practitioners [6-9] have been used regularly for this purpose. Yet, several issues such as the subjectivity of the assessment, small sample sizes and the lack of robust statistical analysis make this approach unreliable [5]. More rigorous morphoscopic ancestry estimation methods such as Optimized Summed Scoring Attributes (OSSA), and Decision Tree Modeling (DTM) have been developed, offering more reliable statistical models for ancestry prediction [10].

As an alternative to the visual assessment of ancestry, metric analysis can identify patterns in skull shape that may not be detected visually. Such analyses may involve single measurements or ratios of two measurements, but the most accepted approach is the application of discriminant functions as first proposed by Giles and Elliot [11] in the 1960s. More recently, the Giles and Eliot approach of classifying individuals to an ancestral group based on their similarity to reference groups has been replaced by a computerised approach; the program FORDISC [12,13]. The operator inserts cranial measurements into the electronic data forms and selects the appropriate groups for comparison. Then, the software produces probabilities of the individual belonging to each selected group. Sex also enters the equation, estimated either by the software based on the cranial measurements or by any other osteological method. The latest version of the software offers the option to create customised equations based on the preservation of a given skull and uses a large comparative database, which includes the Howells database, Terry and Hamann-Todd collections and Forensic Anthropology Data Bank (FDB). A European version of FORDISC is the software COLIPR [14] that includes reference data from modern collections from Prague (Czech Republic), Lisbon and Coimbra (Portugal). An obvious disadvantage of the latter software is the lack of reference populations from the rest of Europe, which would naturally result in erroneous classification of individuals from other ancestral groups. In addition, AncesTrees [15] is freely available software that estimates ancestry based on randomized decision trees. The latter is based on craniometrics variables taken on 1,734 individuals, representative of six major ancestral groups and selected from the Howells' craniometrics series [16].

Similar to the metric approach of ancestry estimation is the application of geometric-morphometric analysis on 3D crania for the quantification of shape through landmark configurations. This approach preserves the physical integrity of an object by summarizing shape through the use of corresponding homologous data points situated in 2- or 3-dimensional space, thereby permitting a more objective, comprehensive, and quantifiable

interpretation of the biological information [17-19]. Geometric morphometric methods are employed in the development of ancestry estimation software known as 3D-ID. 3D-ID integrates the principles of geometric morphometric shape analysis with a large reference database based on inter-geographical variation across numerous regions (n>2000) [20,21].

Stull et al. [22] also used shape variables to separate three ancestral groups of South Africans reaching up to 89% cross-validated classification accuracy. These results were slightly higher, compared to the ones reported for traditional craniometrics variables. Spradley and Jantz [23] compared three methods of ancestry estimation (Geometric-Morphometrics, Standard and Non-standard interlandmark distances) employing FORDISC 3.1 and several other software in a sample consisting of American Blacks, American Whites and Hispanic individuals. The results indicated that Non-standard inter-landmark distances gave the highest classification accuracy. Urbanová et al. [24] tested the accuracy of ancestry and sex assessment using four identification software tools (FORDISC 2.0, FORDISC 3.1.293, COLIPR 1.5.2 and 3D-ID 1.0) in a sample of 174 documented human crania of Brazilian origin belonging to different ancestral groups (i.e., European Brazilians, Afro-Brazilians, Japanese Brazilians, and individuals of admixed ancestry) and reported barely 50% correct classification accuracy. Validation studies clearly demonstrate that software platforms (either inter-landmark distance or landmark-based) have a limited ability to correctly and reliably identify subtle biological intra- and inter-population variation which has not been captured in the reference sample [24-26]. Elliot and Collard [27] also tested FORDISC in a sample of 200 individuals of known ancestry "with and without the test specimen's source population included in the program's reference sample, and with and without specifying the sex of the test specimen" and concluded that no more than 1% of the sample was classified with a high degree of confidence. The results of these validation studies suggest that the existing methods are insufficient to provide answers in many situations where the ancestry of a deceased individual is crucial for identification.

At this point, it is imperative to differentiate ancestry from "race," which, as pointed out by Konigsberg et al. [28], is a socially structured mechanism of self-identification and group membership. Although the term race is scientifically incorrect, it appears frequently in descriptions in a missing persons database. Traditional morphological studies of race started in the US and involved broad categories such as African-American, European-American, American Indian, Hispanic, Native Alaskan etc. but these classifications are problematized by the advanced admixture that is taking place globally as well as inconsistencies in census systems between countries/geographical regions. For example, in Australia, individuals are asked to identify whether they are of indigenous or aboriginal origin when registered at the Census office, while in the UK one has a great selection of subcategories including a number of admixed options [5]. Consequently, ancestry estimation can be a very complicated issue, as, on many occasions, phenotypic expression will not agree with the nationality of the individual. In addition, studies on craniofacial variation in the Balkan [29] and Iberian Peninsula [30] showed considerable craniofacial variation amongst subgroups (e.g. Bosnians vs Croatians). Thus descriptions such as Eastern or Southern European would be insufficient.

It is evident that ancestry estimation can be problematic when the individual can be of any region in the world. Yet, in more restricted geographical areas group assignment can actually be less complicated and more reliable, as for example the Stull et al. study [22] of South Africans. Methods of ancestry estimation do not differ conceptually from the methods employed to estimate other biological information. Furthermore, common sense dictates every case should be considered with regards to its specific geographic context. The current study aspires to develop an ancestry estimation methodology for the identification of missing persons from a recent conflict on the island of Cyprus in 1974 [31].

In July 1974, Turkish forces invaded the northern part of Cyprus in response to a military coup taking place on the island, in attempt to unite the island to Greece. The Greek Cypriot armed forces attempted to resist, and the conflict resulted in a large number of missing persons from both sides. According to the Committee on Missing Persons in Cyprus (CMP) a total of 2002 individuals were recorded missing in 2006 when the effort of recovery, identification and repatriation started. The first bodies were identified and returned to their families in 2007, and, by 2017, 1217 bodies were exhumed and of these 856 identified. Yet, after a decade of intensive efforts by CMP, 864 Greek-Cypriots and 282 Turkish-Cypriots are still missing. The main method of identification is DNA comparison, which is conducted by a contracted US laboratory, while the collection of samples from the families of the missing people are conducted by two laboratories, one for the Greek and one for the Turkish part [31]. It is evident that the estimation of sex and ancestry is an essential step for the biological profiling of the individuals, so that possible matches can be identified. Yet, there are essentially no population specific data for the two groups that can be used to speed the identification process. This study will employ craniometrics variables to explore population differences between Turkey, Cyprus and Greece (Crete), with the objective of developing a classification system of ancestry that can aid forensic identification in the region. At this point it is essential to provide some information on these three neighbour populations.

Greek-Cypriots share the same climate, diet and social structure with Greeks. They speak a Greek dialect and are Christian Orthodox. Genetic studies looking at admixture patterns revealed that 23% of Cypriot DNA carries Greek markers, followed by Iranian (14%) and South Italian (8%). In addition, Cypriots carry smaller percentages of Armenian, Syrian, Georgian, Saudi and Palestinian markers. [32]. The biggest DNA contributors to the modern Greek genome is found to be Polish 30%, followed by Italians (15%), Cypriots (11%) and Iranians (10%) [32]. The Greek sample for this study derives from Crete. Genetic studies support that modern Cretans from Heraklion prefecture are very similar to Minoans [33] which indicates very little admixture with other groups that have settled on the island (including Turkish) in the past 4000 years. Genetic isolation has been mainly attributed to cultural and religious differences. This is also in accordance with biometric studies suggesting that craniofacial characteristics in Greeks remained unaltered for the past 4,000 years [34-36]. Turkish population carries 11% Greek genetic markers followed by 10% Armenian, 9% Iranian markers, 7% Georgian, 7% West Sicilian and 6% Cypriot [32]. A large study of the paternal lineages of Turkish-Cypriots suggested that they had the shortest genetic distance with Lebanon, Turkey and Cyprus followed by Northern Greece and Sicily

[37]. Obviously, sampling has a major effect on the results of such genetic studies, and interpretation of the genetic data should be done with caution.

Although the primary objective is identification of missing persons from the 1974 conflict, the quantification of craniofacial characteristics of each group employed in this study (Greeks, Greek Cypriots and Turkish) will result in population affinity standards that can be used for ancestry estimation in various occasions involving unidentified human remains in the region of the Mediterranean. . The null hypothesis of this study is that the three groups do not differ significantly in their craniofacial characteristics. Rejection of the null hypothesis would mean that the three groups can be successfully separated through statistical operations. The latter would have important implications for the ongoing investigations of mass graves both in Turkey and Cyprus, as well as other forensic investigations of unknown skeletal remains in these regions.

Material and methods

Sample description

The sample consists of 160 dry crania of Greek origin from the Cretan collection [38, 39], 137 dry crania of Greek-Cypriot origin from a cemetery population from Limassol, Cyprus [40] and 380 CT scans of individuals from Turkish origin taken from hospital archives for a study on sexual dimorphism [41]. Demographic information of the sample can be found in Table 1.

Table 1.HERE

CT scans were used as an alternative to dry skulls due to the lack of documented skeletal collection for the Turkish population. Multi-detector CT (MDCT) examinations were performed using a 128-slice MDCT scanner (Siemens Medical Solutions, Erlangen, Germany). All scans were obtained with the patients in supine position, using the following parameters: tube voltage, 120 kV; 150 effective mAs; slice thickness 1 mm. MDCT images were obtained using 3D reconstructions and a volume-rendering technique (VRT). Each measurement was performed by researchers manually using a Leonardo workstation.

To estimate the error between physical and virtual skulls we used 20 randomly selected skulls from the Cretan collection that were scanned for a different project [42].

Data acquisition

Eleven measurements were taken in both dry and virtual skulls. These include Maximum cranial length (CL), Maximum Cranial Breadth (CB), Bizygomatic diameter (BizB), basion-bregma height (Ba-Br), Cranial base length (Na-Ba), left orbital breadth (OrbBL), left orbital length (OrbHL), biorbital breadth (BiorbB), interorbital breadth (IntorbB), foramen

magnum breadth (ForMB) and foramen magnum length (ForML). Description of each measurement can be found in Table 2.

Table 2. HERE

Error estimation

Intra- and inter-observer error was estimated in a sample of N=25 virtual crania in measurements taken by two radiologists with experience in skeletal assessment. In addition, the measurement error between physical and virtual skulls (measured by the same observer) from the Cretan collection was estimated (N=30). Error quantification was done using technical measurement error (TEM), relative TEM (rTEM) and coefficient of reliability (R) of the measurement [22, 43].

Data analysis

Variables were tested for normality (Shapiro-Wilk test) and equal variances (Levene's test) between the two groups (males and females) for each population. Normality was violated in some occasions, thus non-parametric tests (Mann–Whitney U test) were used to explore if there are statistically significant differences between sex groups per population and between the populations per sex group.

Principal components analysis (PCA) was carried out to explore population differences in the cranium of modern Cretans, Greek Cypriots and Turks. The covariance matrix was used in this analysis. PCA extracts maximum variance from a dataset with a few orthogonal components. The first principal component is the linear combination of observed variables that divides the subjects into groups by maximising the variance of their component scores. The second component formed is the linear combination of the observed variables that extract maximum variability uncorrelated to the first component, and the same holds for all components extracted thereafter [44]. The PCs are ordered with the first component extracting the most variance and the last extracting the least variance.

Discriminant function analysis (DFA) was used to create formulae for the estimation of ancestry.

Data analysis was done using SPSS 22.

Results

Intra- and inter-observer error was estimated using technical measurement error (TEM), relative TEM (rTEM) and coefficient of reliability (R) of the measurements. The results are illustrated in Table 3. Intra-observer error falls within acceptable limits for most of the variables (< 5% and >95% for rTEM and R, respectively). Inter-observer error is relatively higher with rTEM values less than 5% while R seems to overpass the 95% threshold for some parameters (Table 3). Interestingly, the variables with the highest error in both cases are OrbHL and OrbBL with R=0.70 between two different observers. The comparison

between physical and virtual measurements resulted in low error rates (rTEM ranging from 0.23 to 4.93) with R values lower than the 95% limit for BizB, OrbHL and IntorbB (Table 3).

Table 3. HERE

Population differences

K-S and S-W tests were used to test for normal distribution of the data for each variable and for the three populations per sex. S-W test revealed several variables that did not follow normal distribution. This however is not expected to cause significant problems in large samples (>40) [45]. Kruskal-Wallis (Monte-Carlo 2-tailed test based on 10,000 subsamples) test for non-parametric data was used to explore differences between populations for each variable. Descriptive statistics and the results of Kruskal-Wallis test for 3 independent samples per sex group can be seen in Table 4. Mean CL is greater in Cretans while CB is greater in the Turkish sample. CL and BiorbB do not seem to differ significantly between the groups.

Table 4 . HERE

To get a better indication of the differences Mann–Whitney U (Monte-Carlo 2-tailed test based on 10,000 subsamples) test was used to explore differences between any two populations. Comparing Greeks and Greek Cypriots, we found that only a few variables differ significantly between the populations, namely CL, Ba-Br and IntorbB for males and OrbHL and InterorbB for females. Differences between Greeks and Turks are significantly greater for both males and females. The same is observed for the Greek Cypriots and Turks comparison. Detailed information on these tests can be found in **supplementary Table 1.**

PCA

Principal component analysis was conducted using 11 variables (**Fig. 1**). The first analysis extracted 3 PC: PC1.1, PC2.1, PC3.1 each accounting for 44%, 21% and 11.9% of the variance in the total sample. PC1.1 and PC2.1 were plotted and labelled according to a) Population, b) Sex. As seen in Plot 1a PC1.1 separates the Turkish sample clearly from the two other samples that seem to cluster very close together. PC2.1 actually separates the sample according to sex as can be seen in Plot 1b.

DFA

Two multivariate discriminant functions were created for each sex group using stepwise DFA. For Males 9 variables (CL, CB, BaBr, BaNa, BiorbB, ForMB, IntorbB, OrbHL, OrbBL) were selected for the model. For females 7 variables (CB, BaBr, BaNa, ForMB, IntorbB, OrbHL, OrbBL) were selected for the model. $F1_m$, $F2_m$, $F1_f$ and $F2_f$ and group centroids for each population and function for both males and females respectively can be seen in Table 5. OrbHL and OrbBL exhibited the largest intra- and inter-observer error in our group of variables thus it was decided to run a second analysis excluding these variables. Analysis 2

was conducted again using the stepwise procedure that selected 5 variables (BaBr, BaNa, CB ForMB, IntorbB) both for males and females.

Table 5. Here

Classification accuracy was calculated per group and in total for both original and cross-validated data. Classification accuracy for cross-validated data ranged from 74.1 to 97.9% (Analysis 1), and it was in all cases very close to the accuracy obtained for the original data. The best classification accuracy was obtained for the Turkish sample, both males and females. In total, classification accuracy reached 89% in males and 85% in females for cross-validated data (Table 6). Discriminant scores were calculated for each individual and plotted as seen in Figure 2. If a female cranium is discovered, 7 measurements should be taken and the DS should be calculated, according to Table 5, for $F1_f$ and $F2_f$. The two coordinates should then be plotted in the Plot 3b and the closest group centroid will indicate the population to which the unknown cranium belongs.

Analysis 2 resulted in 81-83% classification accuracy for cross-validated data (Table 6). The same trend was observed with positive sex bias for the male group. Discriminant scores were calculated for each individual and plotted as seen in Figure 2. If a female cranium is discovered, 5 measurements should be taken, and the DS should be calculated according to Table 5 for F3f and F4f. The two coordinates should then be plotted in the Plot 3b and the closest group centroid will indicate the population to which the unknown cranium belongs.

Table 6. Here

As clearly seen in both DFA and PCA analysis, Cretans and Cypriots cluster very close to each other compared to the Turkish sample. In addition, differences were found only in a few variables according to Mann–Whitney U tests. Thus the Cretan and Cypriot samples were merged, and stepwise DFA was performed again. According to suppl. Table 1, CL was not found to differ significantly between the two new groups (Greeks and Turkish). Thus it was omitted from the analysis. Ba-Pr was again excluded due to small sample size. Classification accuracy reached 96-98% for cross-validation data for females and males, respectively. The new formulae are illustrated in Table 7. Please note that sectioning point is set to zero in all cases.

Table 7. Here

Discussion

Ancestry estimation is unquestionably a major component of reconstructing the biological profile of human remains that require identification. Quantitative methods present greater advantages in terms of methodological robustness compared to morphological methods. They are easier to apply, they are more objective, and experience has less influence on the results. In addition, court requirements mandate the reporting of error rates for any method employed in forensic casework. This framework makes current

ancestry estimation methods, traditional and computer-based, problematic, especially taking into account the low accuracy rates reported by numerous validation studies [24-27]. The main source of bias is the potential lack of the target specimen's source population in the reference sample [24-26]. In controlled conditions, such as in identification of individuals of known nationality from a mass accident or casualties from war zone involving known ethnic groups, this problem is minimised.

The current study employed craniometric variables to explore population differences between three neighbouring countries: Cyprus, Greece and Turkey. The most important objective of the study was to develop population specific standards that can be employed to aid positive identification of Greek Cypriots and Turks that went missing in the 1974 conflict. The forensic framework here limits the possible ethnic groups to two or three (taking into account that Greek nationals may have lived in Cyprus). This creates ideal conditions in which to apply quantitative approaches for ancestry estimation. Sex estimation standards for the Cretan and the Turkish populations have been the subject of other published studies [38, 41]. Thus, they are not the focus of this work. Since cranial features are considered to be highly associated with genetic influences, one would expect craniometrics characteristics of the three groups to reflect their true genetic distance. Yet, history showed continuous variation of ethnic, religious and political changes in this region of the world, with numerous wars, migrations, and constant changes in borders. Given gaps in historical knowledge, demographic structure changes caused by forced migration and the mutual exchange of populations and admixture cannot be totally discounted despite the belief that social and religious differences kept the Greek and Turkish communities apart.

Our study showed that cranial features differ significantly between the three populations, and pairwise comparisons revealed that fewer differences existed between Cretans and Greek-Cypriots. PCA showed Cretans and Cypriots cluster together for both male and female comparisons, while Turkish clusters separately in both cases. DFA analysis using three groups results in 86% and 87% accuracy for cross-validated data. As seen in Table 6, correct classification for Cretans and Cypriots ranges between 72% and 82% while Turkish exhibits over 94% classification accuracy for both sexes. Cypriot males classify better (84%) than females (72%) while Cretans present similar rates for both sexes. Merging Cypriots and Cretans as one sample (Greeks) results in 96-98% accuracy for cross validated data (Table 7).

This study merged traditional osteometric variables with virtual measurements taken in CT scans due to the lack of osteological reference collections for the Turkish population. Comparison of the parameters taken on physical versus virtual skulls indicated acceptable levels of measurement error. Previous research also showed that cranial measurements on dry versus virtual skulls from CT scans exhibited smaller % differences compared to postcranial measurements [22]. The same study reports the highest error for the orbit measurements (OrbBL and OrbHL), which are two of the variables used in this study. Inter-observer agreement for these measurements was also lower compared to the other measurements, but virtual vs. physical measurements showed low agreement only for

OBB (Table 3). To eliminate this potential bias, we excluded OrbBL and OrbHL and repeated the analysis. The classification accuracy was reduced slightly (3-6%) (Table 6 and 7), but the groups were separated successfully. Merging Cypriots and Cretans as one sample (Greeks), results in 91-92% accuracy for cross-validated data.

The results obtained in this study are in agreement with genetic data that suggest higher genetic similarity between Cypriots and Greeks than Turks [32]. The existence of over 20% Greek markers in the Cypriot DNA seems sufficient to produce similarities in cranial morphology. Common markers between Turkish and Greeks/Greek-Cypriots do not exceed 11%, and, perhaps, these do not affect cranial morphology to the same degrees, producing the striking separation of the two groups (Greeks/ Cypriots vs Turks). These results create great confidence in the application of the produced functions in the forensic problem of ancestry estimation in Cyprus, specifically when applied to the unidentified individuals from the 1974 conflict. In addition, these standards can be employed, depending on the circumstances, to other forensic situations where ancestry is an issue but the geographic area of origin is limited in the triangle between Turkey, Cyprus and Greece. It must be stressed though that the samples may not be representative of the whole region of Greece and Turkey, which creates the need for larger validation studies before applying the method on a wider scale.

As expressed throughout the course of this paper, the current needs for positive identification concerning the population samples under study resulted in the development of population-specific standards. We provide here several discriminant functions to assess ancestral group for both male and female individuals of Greek, Greek Cyprus and Turkish origin. Moreover, two functions are also presented for ancestry assessment on Greeks (including Greek Cypriots) and Turkish. Based on the accuracy rates obtained, it seems that the method developed in this paper might be used with confidence for legal investigations, as it reached percentages of correct classification similar to other existing techniques (15,23). Nonetheless, from a forensic standpoint, each method should be evaluated under the scrutiny of the case specifics and applied or rejected accordingly.

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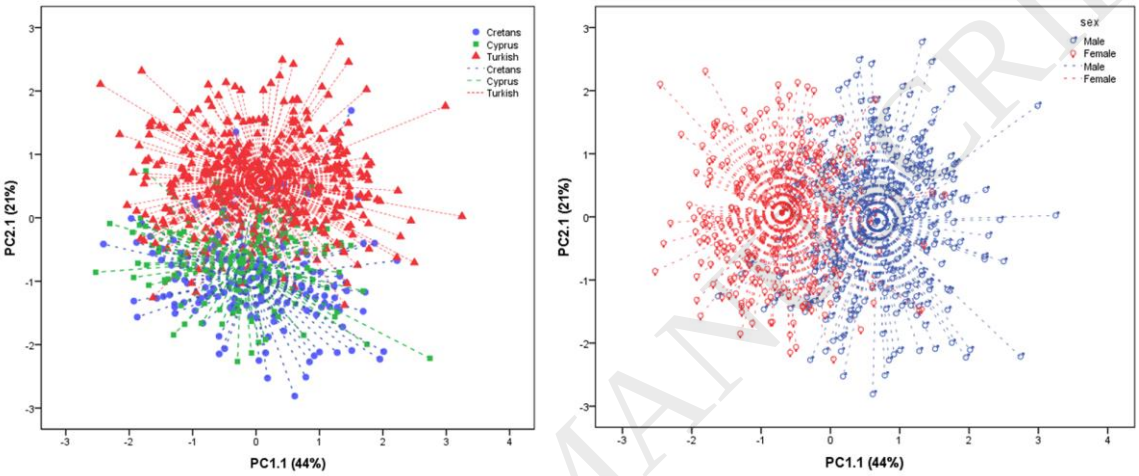
Figure legends

Figure 1. Principal component analysis using 11 variables. Plot of the first two principal components according to population (left) and sex (right).

Figure 2. Discriminant scores for functions 1 and 2 for males (left) and females (right).



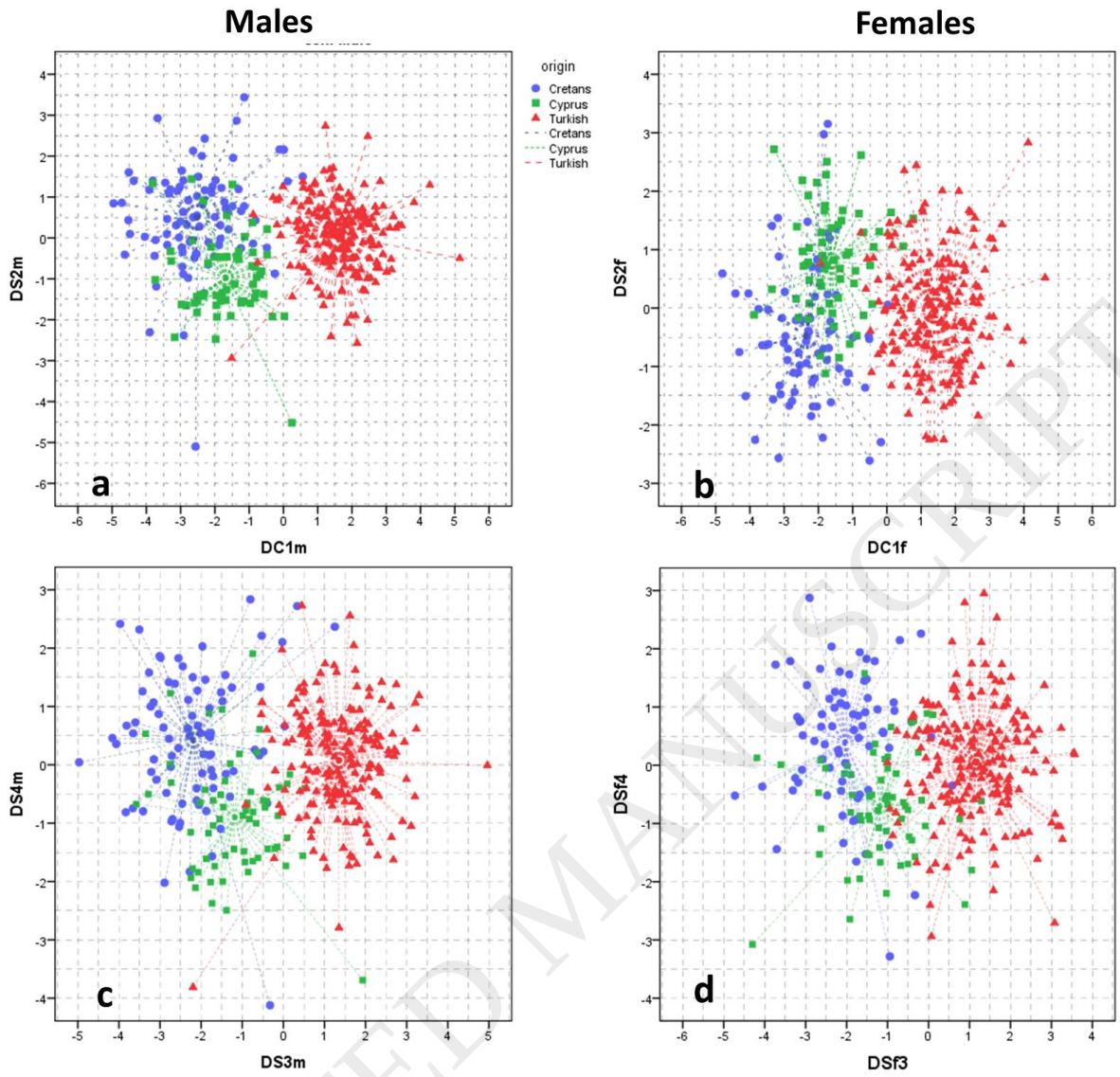


Table 1. Age and sex distribution of the study sample

Sample	Males	Females	Age range	Mean Age	SD
Cretan	85	75	19-98	69.1	15.9
Cypriot	69	68	45-100	76.4	13.3
Turkish	190	190	18-45	30.7	11.2
Total	344	333	18-100	58.7	24.6

Table 2. Anthropometric measurements used in the study

	Measurements	**Distance**
CL	Maximum cranial length	Distance between glabella and opisthocranion
CB	Maximum cranial breadth	Distance between euryon and euryon
BizB	Bizygomatic diameter	Distance between most lateral points on the zygomatic arches
Ba-Br	Basion-bregma height	Distance from the lowest point on the anterior margin of foramen magnum (ba), to bregma
Na-Ba	Cranial base length	Distance from nasion to basion
Ba-Pr	Basion-prosthion length	Distance from basion to prosthion
OrbBL	Left Orbital breadth	Distance from dacryon to ectoconchion
OrbHL	Orbital height	Distance between the superior and inferior orbital margins
BiorbB	Biorbital breadth	Distance between right and left ectoconchion
IntorbB	Interorbital breadth	Distance between right and left dacryon
ForML	Foramen magnum length	Distance from basion to opisthion
ForMB	Foramen magnum breadth	Distance between the lateral margins of foramen magnum at the points of greatest lateral curvature

Table 3. Intra- and Inter-observer error is quantified by calculating TEM, rTEM and R for each variable.

	Intra-Observer Error (N=25)			Inter-Observer Error (N=25)			Measurement error (osteometric-virtual-same observer) (N=30)		
	TEM	rTEM	R	TEM	rTEM	R	TEM	rTEM	R
CL	1.01	0.57	0.99	1.95	1.11	0.97	0.41	0.23	1.00
CB	0.80	0.56	0.98	1.75	1.22	0.89	1.35	0.99	0.95
BizB	0.87	0.69	0.99	2.57	2.03	0.88	2.39	1.87	0.79
BaBr	0.92	0.72	0.98	2.23	1.73	0.90	0.56	0.41	0.99
BaNa	0.73	0.71	0.99	1.59	1.56	0.95	0.38	0.38	0.99
BaPr	0.69	0.72	0.99	2.25	2.37	0.91	0.95	1.03	0.97
OrbBL	0.76	2.15	0.88	1.17	3.31	0.70	1.23	3.30	0.72
OrbHL	0.68	1.88	0.87	1.06	2.91	0.70	0.62	1.88	0.92
BiorbB	0.97	1.02	0.95	1.85	1.95	0.82	0.73	0.76	0.96
IntorbB	0.73	2.80	0.91	1.17	4.45	0.77	1.00	4.93	0.85
ForMB	0.82	2.22	0.91	1.23	3.36	0.79	0.56	1.83	0.97
ForML	0.65	2.06	0.93	1.14	3.62	0.80	0.44	1.26	0.97

Table 4 . Descriptive statistics and Kruskal-Wallis test for 3 independent samples with Monte-Carlo 2-tailed test based on 10000 subsamples

		Variable	CL	CB	BizB	BaBr	BaNa	BaPr	OrbBL	OrbHL	BiorbB	IntorbB	ForMB	ForML
Greek sample	Males	N	85	85	85	85	85	85	85	85	85	85	85	85
		Mean	180.8	137.5	130.3	139.2	101.7	92.85	37.92	33.75	97.68	20.54	31.26	36.24
		SD	6.56	6.37	4.89	6.04	3.80	4.95	2.34	2.34	4.21	2.64	2.76	2.80
	Females	N	75	75	74	75	75	74	75	75	75	75	75	75
		Mean	172.52	133.44	122.27	133.21	96.83	89.67	36.42	33.03	92.98	20.02	28.91	34.62
		SD	5.84	5.38	4.20	5.02	4.57	4.111	1.93	2.10	4.09	2.31	2.61	2.39
Cypriot sample	Males	N	68	69	64	68	67	11	62	62	65	66	67	67
		Mean	178.14	136.71	130.29	136.80	101.34	93.887	37.99	33.90	97.92	23.60	30.63	36.05
		SD	7.10	6.06	5.75	5.86	5.60	5.112	1.70	1.87	3.57	2.72	2.34	2.74
	Females	N	68	68	64	67	66	8	64	62	65	67	67	67
		Mean	171.24	134.01	122.00	131.51	96.64	94.554	36.64	33.89	94.59	22.71	28.86	33.82
		SD	6.82	5.51	5.21	5.58	3.97	11.776	1.86	2.04	6.43	2.21	1.98	2.60
Turkish sample	Males	N	190	190	190	190	190	190	190	190	190	190	190	190
		Mean	179.90	146.63	132.49	131.79	104.46	97.226	35.56	37.18	97.74	25.76	31.72	36.81

		SD	7.85	5.36	5.07	5.60	4.27	5.25	1.63	1.93	3.55	2.15	2.23	2.70
	Females	N	190	190	190	190	190	190	190	190	190	190	190	190
		Mean	171.76	141.29	123.39	125.96	97.77	91.32	34.08	36.02	93.44	24.54	30.11	35.13
		SD	6.63	5.62	4.66	4.93	4.38	5.26	1.85	2.11	3.50	2.05	1.94	2.15
Mean Differences between the 3 samples	Males	Chi-Suare	5.255	156.231	14.737	95.884	35.629	42.375	110.133	141.608	.582	149.199	9.850	5.559
		Asymp. Sig	0.072	**0.000**	**0.001**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	0.748	**0.000**	**0.007**	0.062
		Monte Carlo Sig	0.073[c]	**0.000[c]**	**0.000[c]**	**0.000[c]**	**.000[c]**	**0.000[c]**	**0.000[c]**	**0.000[c]**	0.746[c]	**0.000[c]**	**0.007[c]**	0.060[c]
	Females	Chi-Suare	2.009	107.379	5.199	105.380	4.999	4.802	96.978	105.970	2.951	139.075	27.124	14.927
		Asymp. Sig	0.366	**0.000**	0.074	**0.000**	0.082	0.091	**0.000**	**0.000**	0.229	**0.000**	**0.000**	**0.001**
		Monte Carlo Sig	0.362[c]	**0.000[c]**	0.077[c]	**0.000[c]**	0.085[c]	0.089[c]	**0.000[c]**	**0.000[c]**	0.236[c]	**0.000[c]**	**0.000[c]**	**0.001[c]**

Table 5 . F1m, F2m, F1f and F2f and group centroids for each population and function for both males and females.

	Males		Females	
	F1m	F2m	F1f	F2f
CL	-0.015	0.048		
CB	0.07	0.096	0.076	-0.085
BaBr	-0.089	-0.009	-0.131	0.025
BaNa	0.087	-0.003	0.07	-0.064
BiorbB	-0.106	0.102		
IntorbB	0.243	-0.41	0.162	0.416
ForMB	0.069	0.146		
ForML			0.068	-0.209
OrbBL	-0.212	-0.254	-0.311	0.231
OrbHL	0.239	0.039	0.224	0.035
Constant	-2.612	-17.581	-3.465	2.986
Functions at Group Centroids				
Cretans	-2.521	0.509	-2.359	-0.469
Cyprus	-1.686	-0.949	-1.604	0.748
Turkish	1.633	0.057	1.409	-0.046

Table 6 . Classification accuracy for original and cross-validated data.

sex	accuracy			Cretans	Cyprus	Turkish	Total
Male	Original	Count	Cretans	66	15	4	
			Cyprus	10	48	0	
			Turkish	1	3	186	
		%	Cretans	77.6	17.6	4.7	90.1
			Cyprus	17.2	82.8	0	
			Turkish	0.5	1.6	97.9	
	Cross-validated	Count	Cretans	63	18	4	
			Cyprus	10	48	0	
			Turkish	1	3	186	
		%	Cretans	74.1	21.2	4.7	89.2
			Cyprus	17.2	82.8	0	
			Turkish	0.5	1.6	97.9	
Female	Original	Count	Cretans	57	16	2	
			Cyprus	12	47	2	
			Turkish	1	11	178	
		%	Cretans	76	21.3	2.7	86.5
			Cyprus	19.7	77	3.3	
			Turkish	0.5	5.8	93.7	
	Cross-validated	Count	Cretans	55	18	2	
			Cyprus	14	44	3	
			Turkish	1	11	178	
		%	Cretans	73.3	24	2.7	85.0
			Cyprus	23	72.1	4.9	
			Turkish	0.5	5.8	93.7	

Table 7 . Discriminant Functions for Greeks and Turkish

	Variables	F1	GR	TU	Total	Classification	
Males	BaBr	-0.089	138	187	325	N	Original
	BaNa	0.079	96.5	98.4	97.6	%	
	BiorbB	-0.092	138	187	325	N	Cross-validated
	CB	0.081	96.5	98.4	97.6	%	
	ForMB	0.087					
	IntorbB	0.181					
	OrbBL	-0.239					
	OrbHL	0.238					
	(Constant)	-5.344					
	Variables	F2	GR	TU	Total	Classification	
Females	BaBr	-0.128	129	184	313	N	Original
	BaNa	0.075	94.9	96.8	96	%	
	CB	0.080	128	184	312	N	Cross-validated
	ForMB	0.103	94.1	96.8	95.7	%	
	IntorbB	0.114					
	OrbBL	-0.331					
	OrbHL	0.208					
	(Constant)	-2.944					